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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/528,846	02/09/2006	Tatsuo Hoshino	21414 US C0381435/0186340	2015
7590 12/05/2006			EXAMINER	
Stephen M Haracz Bryan Cave 1290 Avenue of the Americas New York, NY 10104			CHOWDHURY, IQBAL HOSSAIN	
			ART UNIT	PAPER NUMBER
			1652	

DATE MAILED: 12/05/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

T4

Office Action Summary	Application No. 10/528,846	Applicant(s) HOSHINO ET AL.	
	Examiner Iqbal Chowdhury, Ph.D.	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☐ Responsive to communication(s) filed on ____.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-7 is/are pending in the application.
 4a) Of the above claim(s) ____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) ____ is/are allowed.
- 6) ☒ Claim(s) 1-7 is/are rejected.
- 7) ☐ Claim(s) ____ is/are objected to.
- 8) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) ☒ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____. |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date <u>04/05</u> . | 6) <input type="checkbox"/> Other: ____. |

DETAILED ACTION

This application is a 371 of PCT/EP03/10294.

Claims 1-7 are pending and are present for examination.

Priority

Acknowledgement is made of applicants claim for foreign priority of EPO 02021600.8 of 9/27/2002.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 3/23/2005 was filed with the National Stage Application. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Drawings

There is no drawing with this application.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In the present instance, claim 1 recites the “ β -carotene ketolase gene and belonging to the genus *Xanthophyllomyces* (Phaffia)” which is unclear as written as it

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sounds like the β -carotene ketolase gene must be from *Phaffia* and not that the microorganism is a *Phaffia* strain.

Claim 2 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In the present instance, claim 2 recites the "mutant thereof" which is unclear as to the scope of recombinant microorganism that is encompassed. In another words, how many changes can be made to the microbial strain and still be a "mutant thereof".

Claim 3 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In the present instance, claim 3 is confusing as it includes E-396 (FERM BP-4283) as a source of β -carotene ketolase gene but claim 1 from which it depends recites that the β -carotene ketolase gene must be from *Agrobacterium*, *Alcaligenes*, *Paracoccus* or *Haematococcus*. As the E-396 (FERM BP-4283) is not disclosed as from any of these genera, claim 3 includes subject matter excluded from claim 1.

Claim 4 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 4 is indefinite and vague in the recitation of the "substantially homologous thereto" as it is unclear how homologous to SEQ ID NO: 2 a sequence must be to be encompassed by the phrase "substantially homologous thereof".

Claim 5 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant

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regards as the invention. Claim 5 is indefinite and vague in the recitation of the “control sequence”. Does “control sequence” mean control regulatory sequence or something else?

Claim 5 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite and vague for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 5 is indefinite and vague as recitation “the control sequence” lacks antecedent basis.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 1 is directed to a process for producing canthaxanthin and echinenone, which comprises cultivating a recombinant microorganism belonging to the genus *Xanthophyllomyces* (*Phaffia*) which is expressing any β -carotene ketolase gene, which accumulates β -carotene in the aqueous nutrient medium under aerobic conditions, and isolating the resulted carotenoids from the cells of said recombinant microorganism or from the cultured broth, wherein the β -carotene ketolase gene is originated from *Agrobacterium*, *Alcaligenes*, *Paracoccus* and *Haematococcus*. Claim 2 recites the recombinant microorganism is derived from *Xanthophyllomyces dendrorhous*

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(*Phaffia rhodozyma*) ATCC96815, or a mutant thereof and claim 4 recites the microorganism wherein β -carotene ketolase gene is selected from the group consisting of *Agrobacterium aurantiacum*, *Alcaligenes* PC-I, *Paracoccus marcusii* MHI, a gram-negative bacterium E-396 (FERM BP-4283), and *Haematococcus pluvialis*. Claim 5 recites the process wherein the β -carotene expressed in the recombinant microorganism having the control sequences and claim 6 recites the process, wherein the cultivation is carried out at a pH in the range of from 3 to 7 and at a temperature in the range of from 15 to 26°C for 24 to 500 hours. Claim 7 recites that the process, wherein the cultivation is carried out at a pH range of from 5 to 7 and at a temperature in the range of from 18 to 22°C for 48 to 350 hours.

The specification teaches the structure of only a single representative species of such β -carotene ketolase genes. While claims 3 add some additional characteristics to the limitations of the genus of claim 1 i.e. gene is derived from *Alcaligenes* PC-1 (claim 3), none of the characteristics alone is sufficient to change the fact that the claims include gene encoding proteins which are highly variable in both structure and functions as all of the characteristics can be present in proteins of any structure and neither genus recites enough characteristics that a skilled artisan would reasonably expect that any protein having these characteristics would necessarily be highly structurally similar to the disclosed species. Moreover, the specification fails to describe any other representative species by additional identifying characteristics or properties other than the functionality of the gene encoding polypeptide of β -carotene ketolase. Given this lack of description of representative species encompassed by the genus of genes used in the claim, the specification fails to sufficiently describe the claimed invention in such full,

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clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a process for producing canthaxanthin and echinenone by using a recombinant microorganism of *Phaffia rhodozyma* expressing β -carotene ketolase gene from *Alcaligenes* strain PC-1 (GenBank Accession No. D58422), does not reasonably provide enablement for a process for producing canthaxanthin and echinenone by using any β -carotene ketolase gene from any source. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claim 1 is so broad as to encompass a process for producing canthaxanthin and echinenone by using any β -carotene ketolase gene by culturing and expressing in any strains of *Xanthophyllomyces*. Claim 2 recites the recombinant microorganism is derived from *Xanthophyllomyces dendrorhous* or *Phaffia rhodozyma* ATCC96815, or a mutant thereof and claim 4 recites the microorganism wherein β -carotene ketolase gene is selected from the group consisting of *Agrobacterium aurantiacum*, *Alcaligenes* PC-I, *Paracoccus marcusii* MHI, a gram-negative bacterium E-396 (FERM BP-4283), and *Haematococcus pluvialis*. Claim 5 recites the process wherein the β -carotene ketolase gene expressed in the recombinant microorganism using control sequences and claim 6 recites the process, wherein the cultivation is carried out at a pH in the range of from 3 to 7 and at a temperature in the range of from 15 to 26°C for 24 to 500 hours. Claim 7 recites the process, wherein the cultivation is carried out at a pH range of from 5 to 7 and at a temperature in the range of from 18 to 22°C for 48 to 350 hours.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of β -carotene ketolase gene broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the nucleotide and encoded amino acid sequence of only one β -carotene ketolase gene.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple point mutations or substitutions.

The specification does not support the broad scope of the claims which encompass a process for producing canthaxanthin and echinenone by using any β -carotene ketolase gene because the specification does not establish: (A) regions of the protein structure which may be modified without effecting β -carotene ketolase activity; (B) the general tolerance of β -carotene ketolase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any β -carotene ketolase residues with an expectation of obtaining the desired

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biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any β -carotene ketolase gene. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of any β -carotene ketolase gene having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

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invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-7 are rejected under 35 U.S.C. 103 (a) as being obvious over Misawa et al. (GenBank Accession No. D58422, *Alcaligenes* sp. crtW and crtZ genes for beta-carotene hydroxylase and beta-Carotene ketolase, complete cds, created on 7/22/1995), Misawa et al. (Canthaxanthin biosynthesis by the conversion of methylene to keto groups in a hydrocarbon beta-carotene by a single gene, *Biochem Biophys Res Commun.* 1995 Apr 26; 209(3): 867-76, see IDS) in view of Hoshino et al. (US Patent 6,365,386 B1, issue date 4/2/2002, see IDS).

Misawa et al. (GenBank) teach isolation of a beta-carotene ketolase (crtW) gene from *Alcaligenes* PC-1 strains, which is 100% identical to the said gene disclosed by the instant application. Misawa et al. (GenBank) do not teach the use of the gene for a process for producing canthaxanthin and echinenone.

Misawa et al. (BBRC) teach a method of producing canthaxanthin and echinenone from beta-carotene by using a recombinant *E. coli* comprising beta-carotene ketolase gene (crtW) from *Alcaligenes* PC-I. Misawa et al. also teach cloning said gene in expression vector, which is under the control of a promoter, transform an *E. coli* and culturing the said recombinant microorganism at 28°C and produced canthaxanthin and echinenone. Misawa et al. do not teach the use of transformed *Phaffia rhodozyma* by the said gene for producing canthaxanthin and echinenone.

Hoshino et al. disclose a process for producing astaxanthin from beta-carotene in *Phaffia rhodozyma* ATCC96815 comprising all the genes required to produce astaxanthin i.e. beta-carotene ketolase (crtW) and crtZ gene and produce all the intermediates including canthaxanthin, and echinenone as well as astaxanthin (see col.4 2nd par.). Hoshino et al. also

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disclose to use a mutant strain of *Phaffia rhodozyma*, which is blocked for the astraxanthin production i.e. this mutant strain will produce mainly canthaxanthin, and echinenone. Hoshino et al. further disclose the process, wherein the cultivation of the recombinant microorganism is performed at 20°C for over-night (about 24 hr) at a pH wherein the recombinant microorganisms grow well. Hoshino et al. do not disclose the isolation of the beta-carotene ketolase gene from *Alcaligenes PC-1* (claim 5) and expression of beta-carotene ketolase in said recombinant microorganism using control sequences or culturing the recombinant microorganism for 48-350 hours.

It would have been obvious to one of ordinary skill in the art at the time of the invention was made to combine the teaching of Misawa et al. (GenBank), Misawa et al. (BBRC) and Hoshino et al. to produce canthaxanthin and echinenone from beta-carotene by using the beta-carotene ketolase gene (*crtW*) of Misawa et al. (GenBank) to transform the *Phaffia rhodozyma* of Hoshino et al. to produce canthaxanthin and echinenone by using the methods of Hoshino et al.

One of ordinary skill in the art would have been motivated to use *Phaffia rhodozyma* instead of *E. coli* to produce canthaxanthin and echinenone because *Phaffia*, red yeast usually used for the microbiological production systems for natural astaxanthin comprises sufficient amount of natural substrate beta-carotene. One of ordinary skill in the art would have a reasonable expectation of success because using recombinant *Phaffia rhodozyma* for producing canthaxanthin and echinenone is customary and widely used in the art for the biosynthesis of xanthophylls such as canthaxanthin, echinenone, astraxanthin and zeaxanthin from beta-carotene.

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Conclusion

Status of the claims:

Claims 1-7 are pending.

Claims 1-7 are rejected.

No claim is in condition for allowance.


Any inquiry concerning this communication or earlier communications from the examiner should be directed to Iqbal Chowdhury whose telephone number is 571-272-8137. The examiner can normally be reached on 9:00-5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 703-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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